



UNIVERSITI PUTRA MALAYSIA

***DETECTION AND MOLECULAR CHARACTERIZATION OF MYCOPLASMA
GALLISEPTICUM AND MYCOPLASMA SYNOVIAE FROM COMMERCIAL
CHICKENS IN MALAYSIA***

KARTINI AHMAD

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**MASTER OF VETERINARY SCIENCE
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FROM COMMERCIAL CHICKENS IN MALAYSIA**



By

KARTINI AHMAD

© This thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Veterinary Science

May 2012

Specially dedicated to:

**My dearest parents
who always encourage and support me throughout my study,
Azreen and to all family members.**

Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Veterinary Science

**DETECTION AND MOLECULAR CHARACTERIZATION OF
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By

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May 2012

Chairman: Professor Datin Paduka Aini Ideris, PhD

Faculty: Veterinary Medicine

Mycoplasma gallisepticum (MG) and *Mycoplasma synoviae* (MS) are well-known pathogens of poultry, which are distributed world-wide and significantly important for the poultry industry. Both pathogens are capable causing respiratory and joint diseases in chickens and turkeys, subsequently leading to poor production due to poor growth and increase in mortality. Currently, studies on the prevalence of MG and MS, especially on concurrent infections with MG and MS, in commercial chickens as well as genetic diversity of MG and MS isolates are still lacking in Malaysia. Hence, the present study was to detect the presence of MG and MS from commercial chickens (broiler, breeder, layer and village chickens) in Peninsular Malaysia using isolation and molecular methods; and to characterized multigene families of MG (*pvpA*, *gapA*, *mgc2*, *pMGA*, *crmA*³ and *crmC* genes) and MS (*vlhA* gene) based on gene-targeted sequencing (GTS) analysis. A total of 814 samples of choanal slit and trachea swabs from 27 chicken farms within

Peninsular Malaysia were taken from 472 commercial broiler, 105 broiler breeder, 131 layer and 106 village chickens. *Mycoplasma gallisepticum* was detected in three out of four types of commercial chickens farms (broiler, broiler breeder and village chickens), while MS were detected in all four commercial chickens farms (broiler, broiler breeder, layer and village chickens). Overall, both IFA and PCR methods detected high prevalence of MG infection (9.1% and 24.2%, respectively) than MS infection (2.0% and 5.7%, respectively), while concurrent infection of MG and MS (0.7% and 2.5%, respectively) was the least detected in this study. The prevalence of MG infection and concurrent MG and MS infections were detected higher in broiler chickens followed by broiler breeder, layer and village chickens. *Mycoplasma synoviae* was detected higher in broiler breeder chickens followed by broiler, layer and village chickens. These indices indicate that MG and MS are still persisting in commercial poultry production under current biosecurity and disease control programme. The analysis of multigene families of 20 MG field isolates on six cytoadhesin genes revealed their G+C content within 27% - 50% and showed 91-100% sequence homology with MG isolates from USA, Israel, Australia and Russia. Classification of field MG isolates based on a close relationship on nucleotide sequence analysis, sequence similarity identity matrix and phylogeny relations, revealed eleven (11) Malaysian MG field isolates were successfully grouped into 3 categories: 1) S6 strain, 2) ts-11 strain and 3) local field strain. Five isolates (KPR44 L, KPR16W44 L, THNG8W L, PF3H Br and PF7U Br), under local field strain category, were classified as Malaysian isolate as they were more diversified than the reference and published isolates. Analysis on eight MS local isolates

based on size of proline-rich repeat (PRR) region of the *vlhA* gene revealed gene size polymorphism with 41 - 43% G+C content and 95 - 98% sequence homology with Australian and USA isolates. The lengths of PRR encoding sequence of local isolates were within 19 - 35 amino acids and the isolates were classified according to PRR grouping (group A - E). Two field isolates (ALNH5 BB and LHMN L) were classified in group C with 32 a.a. and one isolate (JBSJ Br) was classified in group E with 19 a.a. However, five local isolates (KSVC4 VC, KSVC5 VC, LGW34H9 BB, PPB07 Br and ALNH6 BB) with 35 amino acid (a.a.) size were not classified within the PRR classification as there is no classification on 35 a.a. been published or recorded. These new findings show the unique entity of Malaysian MG and MS field isolates compared to reference and other published isolates.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

PENGESANAN DAN PENCIRIAN MOLEKULAR *MYCOPLASMA GALLISEPTICUM* DAN *MYCOPLASMA SYNOVIAE* DARI AYAM KOMERSIL DI MALAYSIA

Oleh

KARTINI AHMAD

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Mycoplasma gallisepticum (MG) dan *Mycoplasma synoviae* (MS) adalah patogen unggas yang terkenal, yang tersebar di seluruh dunia dan mempunyai kepentingan ketara dalam industri dan ekonomi unggas. Kedua-dua pathogen ini mampu menyebabkan penyakit pernafasan dan sendi pada ayam dan ayam belanda, seterusnya membawa kepada penurunan taraf pengeluaran yang disebabkan oleh penurunan kadar pertumbuhan dan peningkatan kematian. Sehingga kini, kajian prevalens MG dan MS, terutamanya jangkitan serentak MG dan MS, pada ayam komersil serta kepelbagaian perincian genetik isolat-isolat MG dan MS masih kurang di Malaysia. Oleh itu, kajian ini adalah untuk mengesan kehadiran MG dan MS dari ayam komersial (ayam pedaging, ayam pembiak baka pedaging, ayam penelur dan ayam kampung) di Semenanjung Malaysia dengan menggunakan kaedah pengasingan dan molecular; dan mencirikan keluarga multigen MG (gen-gen *pvpA*, *gapA*, *mgc2*, *pMGA*, *crmA*³ dan *crmC*) dan MS (gen *vlhA*) berdasarkan analisis jujukan sasaran (GTS). Sebanyak 814

sampel calitan celahan lelangit dan trakea dari 27 ladang ayam di Semenanjung Malaysia telah diambil daripada 472 ayam pedaging, 105 ayam pembiak baka pedaging, 131 ayam penelur dan 106 ayam kampung. *Mycoplasma gallisepticum* telah dikesan pada tiga daripada empat jenis ayam komersial (ayam pedaging, ayam pembiak baka pedaging dan ayam kampung), manakala MS telah dikesan pada kesemua empat jenis ayam komersial (ayam pedaging, ayam pembiak baka pedaging, ayam penelur dan ayam kampung). Secara keseluruhan, kedua-dua kaedah IFA dan PCR mengesan prevalens jangkitan MG yang tinggi (masing-masing 9.1% dan 24.2%) berbanding jangkitan MS (masing-masing 2.0% dan 5.7%), manakala jangkitan serentak MG dan MS (masing-masing 0.7% dan 2.5%) adalah paling kurang dikesan dalam kajian ini. Prevalens jangkitan MG dan jangkitan serentak MG dan MS dikesan lebih tinggi pada ayam pedaging, diikuti dengan ayam pembiak baka daging, ayam penelur dan ayam kampung. *Mycoplasma synoviae* pula dikesan lebih tinggi pada ayam pembiak baka pedaging diikuti dengan ayam pedaging, ayam penelur dan ayam kampung. Indeks ini menunjukkan MG dan MS masih berterusan dalam produksi ayam komersial di bawah program biosekuriti dan kawalan penyakit yang terkini. Analisis keluarga multigen dari 20 isolat-isolat tempatan MG pada enam gen 'cytadhesin' menunjukkan kandungan G+C adalah dalam lingkungan 27-50%, dengan persamaan jujukan homologi antara 91-100% dengan isolat-isolat MG dari Amerika Syarikat, Israel, Australia dan Rusia. Pengklasifikasi isolat-isolat tempatan MG adalah berdasarkan hubungan rapat pada analisis jujukan nukleotida, keserupaan matriks identiti jujukan dan hubungan filogeni. Ia menunjukkan sebelas (11)

isolat-isolat MG Malaysia telah berjaya dikumpulkan ke dalam 3 kategori: 1) strain S6, 2) strain ts-11 dan 3) strain isolat tempatan. Lima isolat yang dikaji (KPR44 L, KPR16W44 L, THNG8W L, PF3H Br dan PF7U Br), dibawah kategori strain isolat tempatan, telah diklasifikasikan sebagai isolat Malaysia memandangkan ia lebih pelbagai berbanding isolat-isolat rujukan dan terbitan. Analisis pada lapan isolat-isolat tempatan MS berdasarkan saiz kawasan ulangan kaya-prolin (PRR) pada gen *vlhA* mendedahkan saiz gen 'polymorphism' dengan kandungan G+C 41-43%, dan jujukan homologi 95-98% terhadap isolat-isolat Australia dan Amerika Syarikat. Panjang jujukan pengekodan kawasan PRR pada isolat-isolat tempatan adalah dalam lingkungan 19-35 asid amino. Dua isolat tempatan telah diklasifikasikan dalam kumpulan C dengan 32 a.a. dan satu isolat telah diklasifikasikan dalam kumpulan E dengan 19 a.a. Manakala, lima isolat tempatan (KSVC4 VC, KSVC5 VC, LGW34H9 BB, PPB07 Br dan ALNH6 BB) dengan saiz asid amino (a.a.) 35 tidak dikelaskan lagi dalam pengkelasan PRR, memandangkan tiada lagi klasifikasi untuk 35 a.a. diterbitkan atau direkod. Penemuan baru ini menunjukkan entiti unik isolat-isolat MG dan MS Malaysia berbanding dengan isolat-isolat rujukan dan terbitan lain.

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I certify that a Thesis Examination Committee has met on 18 May 2012 to conduct the final examination of Kartini binti Ahmad on her thesis entitled "Detection and Molecular Characterization of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* from Commercial Chickens in Malaysia" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Veterinary Science.

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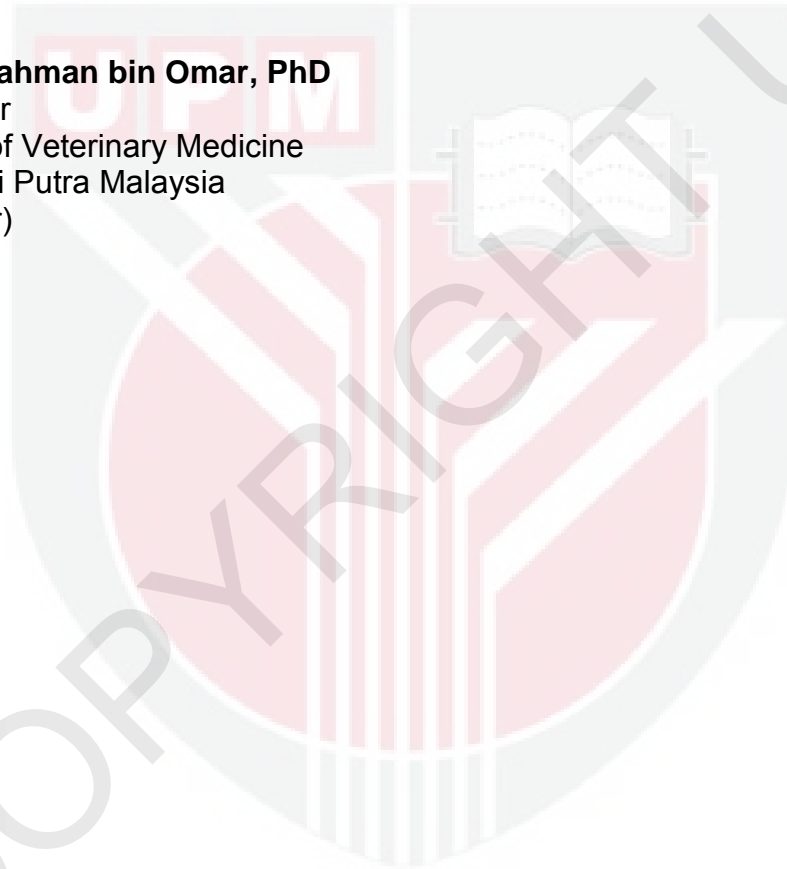
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.



KARTINI AHMAD

Date: 18 May 2012

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